Bio

sequence that prime in opposite directions, and have a particular restriction enzyme site between them, *e.g.*, the left or right border Ti sequences. In the method, chromosomal DNA is digested with a restriction endonuclease and ligated into a circularized DNA molecule. The resulting population of ligated molecules is comprised of a complex mixture of chromosomal DNA and chromosomal-vector DNA hybrids. The plasmid derived region of the hybrid molecules provides the downstream priming site for PCR amplification. The upstream primer may be specific for the vector, or a gene-specific primer. See, *e.g.*, Novak, J and Novak, L, *Promega Notes Magazine* Number 61:27, 1997.—

The paragraph beginning at page 10, line 6 of the application as filed, has been replaced with paragraph revised as follows:

--Random expression of native genes may also be achieved by introduction of a nucleic acid construct comprising a transposon into the genome of interest. Exemplary transposons such as Ac, Ds, Mu or Spm are elements which can insert themselves into genes and cause unstable mutations. The mutations are unstable due to subsequent excision of the transposon from the mutant locus during plant or seed development. (See, *e.g.*, Doring, H. P. and Starlinger (1986), Ann. Rev. Genet. 20:175-200; Federoff, N. (1989), "Maize Transposable Elements" in Mobile DNA. Wowe, M. M. and Berg, D. E., eds., Amer. Soc. Microbiol., Wash., D.C., pp. 377-411.) An exemplary transposon-tagging strategy used to identify a semi-dominant mutation affecting plant height, hypocotyl elongation, and fertility has been described. See, Wilson K. *et al.*, *Plant Cell* 8(4):659-71, 1996.--

The paragraph beginning at page 11, line 21 of the application as filed, has been replaced with paragraph revised as follows:

--Binary Ti-based vector systems are used to transfer and confirm the association enhanced expression of a given gene with the modified trait or phenotype of the plant. Appropriate vectors for this aspect of the invention include plasmids containing at least one T-DNA border sequence (left, right or both), restriction endonuclease sites for the addition of one or more heterologous nucleic acid sequences, adjacent flanking T-DNA border sequence(s), a heterologous nucleic acid sequence (*i.e.*, the coding sequence of identified and isolated genes), operably linked to appropriate regulatory sequences and to the directional T-DNA border sequences, a selectable marker which is functional in plant cells, a heterologous Ti-plasmid promoter, an *E. coli* origin of replication.--

IN THE CLAIMS:

Please cancel claim 13, amend claims 1, 2, 4-10, 12, 14-17 and 19 and replace claims 1, 2, 4-10, 12, 14-17 and 19 with the rewritten claims, as set forth below. Also enclosed,

starting on a separate page following this response, is a marked-up copy of the amended claims showing all changes relative to the previous version.

- --1. (Amended) A method for identifying genes associated with a desired trait in a fruitbearing plant comprising:
- (i) transforming cells of a plant with a plant cell expression vector having an *E. coli* origin of replication, an enhancer, a selectable marker-encoding nucleotide sequence operably linked to a promoter effective to express the selectable marker encoding sequence, a transcription termination element for said selectable marker-encoding nucleotide sequence, and a T-DNA sequence, wherein said transforming cells is by introduction of *Agrobacterium tumifaciens* into hypocotyl or shoot tip tissue derived from said plant in the absence of feeder cells, in a manner effective to express said selectable marker-encoding nucleotide sequence;
- (ii) selecting plant cells which have been transformed by their ability to grow in the presence of an amount of selective agent that is toxic to non-transformed plant cells;
 - (iii) regenerating transformed plant cells to yield mature plants;
 - (iv) selecting plants having a desired trait; and
- (v) identifying, isolating and characterizing genes the transcription of which was enhanced by said element which functions to enhance gene expression.
 - 2. (Amended) The method of claim 1, further comprising the steps of
 - (vi) preparing a separate heterologous gene construct for each isolated gene;
- (vii) transforming plants with said separate heterologous gene construct wherein expression of the isolated gene is enhanced in said plants;
 - (vii) selecting plants having the desired trait.
- 4. (Amended) The method of claim 3, wherein said CaMV 35S enhancer element is a 4X tandem duplicated CaMV 35S enhancer element having the sequence presented as SEQ ID NO:1.

B14

5. (Amended) The method of claim 3, wherein said Figwort Mosaic Virus (FMV) promoter sequence is the promoter sequence presented as SEQ ID NO:5 or the enhancer sequence presented as SEQ ID NO:6.

6. (Amended) The method of claim 3, wherein said peanut chlorotic streak caulimovirus full-length transcript (PCISVFLt) sequence is the enhancer sequence presented as SEQ ID NO:7.

7. (Amended) The method of claim 3, wherein said mirabilis mosaic virus (MMV) promøter sequence is the promoter sequence presented as SEQ ID NO:8.

- 8. (Amended) The method of claim 1, wherein said selectable marker-encoding nucleotide sequence encodes a polypeptide which confers herbicide-resistance to transformed plant cells expressing said marker.
- 9. (Amended) The method of eaim 1, wherein said selectable marker-encoding nucleotide sequence encodes an antibiotic resistance gene which confers resistance to an antibiotic selected from the group consisting of kanamycin, G418, bleomycin, hygromycin, chloramphenicol, ampicillir and tetracycline.
 - 10. (Amenced) The method of claim 9, wherein said antibiotic is kanamycin.

BIS

- 12. (Amended) The method of claim (11, wherein said dwarf plant is a tomato plant.
- 14. (Amended) The method of claim 13, wherein said transforming cells is by introduction of *Agrobacterium tumifaciens* into hypocotyl tissue derived from said dwarf tomato plant.
- 15. (Amended) The method of claim 13, wherein said transforming cells is by introduction of *Agrobacterium tumifaciens* into shoot tip tissue derived from said dwarf tomato plant.
- 16. (Amended) The method of claim 1, wherein said desired trait is a biochemical modification of a plant and fruit selected from the group consisting of a change in the level of, vitamins, minerals, elements, amino acids, carbohydrates, lipids, nitrogenous bases, isoprenoids, phenylpropanoids and alkaloids.
- 17. (Amended) The method of Jaim 1, wherein said desired trait is a fruit-bearing plant specific trait selected from the group consisting of increased resistance to fungal pathogens,

< A P